Remarks/Arguments

Amendment to the Specification:

The Specification was amended to recite priority data for the instant application according to the Examiner's suggestion. No new matter was added and entry of the amendment to the specification is requested.

Amendments to the Claims:

Claim 1 was amended to more clearly define the invention. Support for the amendments to claim 1 may be found throughout the specification and, in particular, may be found in original claim 1, on page 31, lines 25-28, page 3 lines 23-24, and in Example 4. No new matter has been added and entry of the claim amendment is requested.

Priority:

The Final Office Action of May 23, 2005 alleged that the instant application has not complied with one or more conditions for receiving priority because the Declaration did not recite that the instant application is the national stage of International Application Number PCT/GB00/00873 filed March 10, 2000. Applicants have amended the specification to make this recitation. Therefore, this basis of objection is now moot in view of Applicants' amendment and should be withdrawn.

35 U.S.C. 103:

Claims 1-13 stand rejected under 35 U.S.C. 103 as allegedly obvious in view of Ross (WO 91/06678) and Williams (US 6,255,083). Applicants respectfully traverse.

Applicants' have amended claim 1 to more clearly define the invention. Briefly, the claimed invention, as recited in amended claim 1, is directed to a method of sequencing comprising the following steps: First, a plurality of polymerases is immobilized to reaction centers on a solid support wherein each reaction center contains only one polymerase and each reaction center is located at an optically resolvable distance from other reaction centers (step a). Second, a nucleic sample is provided to the polymerase and the nucleic acid sample is hybridized to an oligonucleotide primer (step b). In the third step, four different nucleotides, each differentially labeled and containing a blocked 3' ends are provided so that the polymerase can

attach one of the four nucleotides to the primer by elongation (step c). Because each nucleotide contains a detachable label, and the nucleotide is attached to the primer on a solid support, the detachable label is attached to the solid support after step c. Free nucleotides are removed from the sample (step d) and the label, still attached to the solid support, is detected (step e). The blocking group forming the blocked 3' ends and the label are separated from the incorporated nucleotide (step f) and removed (step g) and their removal is confirmed (step h). Then, steps c to g is repeated until elongation stops (i.e., no new nucleotides are added or the 3' blocking group cannot be removed).

1. There is no motivation to combine Ross and Williams.

There is no motivation to combine Ross and Williams because the two references employ incompatible detection techniques. Ross is directed to a method of sequencing by synthesis where multiple identical copies of DNA templates are used (Ross page 7 lines 1-2). Ross' method involves synthesizing, in the presence of a multitude of identical copies of template DNA, a complimentary strand of nucleic acid. This synthesis is carried out using dNTPs in a stepwise serial manner to build up "numerous copies" of the complementary strand, dNTP by dNTP (Ross, page 7, lines 1-14). As each dNTP is added to the growing complementary molecule, it is identified by the label attached to the dNTPs (Id). Since "numerous copies" of complementary strand is produced, an equally high number of labeled dNTPs are incorporated into the growing complementary strand. Importantly, Ross' method employ as much as 0.1 moles (Ross, page 38, line 12) of templates which could, in turn, incorporate an equal number of labels. Since Ross' templates incorporated a significant plurality of labels, Ross' detection method is also tailored towards multiple label detection (Ross, page 13 and Example 4). Significantly, Ross does not disclose or suggest a method of detecting a single label molecule.

In contrast to Ross, Williams is directed to a method of sequencing which requires the detection of single labels in solution. In Williams' method, individual fluorogenic NTP molecules are identified one at a time as a polymerase incorporates the NTP into an extension product (Williams, column 4 lines 4-22). Williams' NTPs contain a fluorescence quencher and a fluorescent dye. Since the dye is proximate to the quencher on the same NTP, Williams' NTPs are not fluorescent. As Williams NTPs are incorporated, the pyrophosphate-dye moiety on the NTP is separated from the quencher and becomes free in solution and fluorescent (Id.). This fluorescent dye is detected in solution - away from any solid support. (See, e.g., Williams,

column 12, line 11-12 which states "[t]his invention requires the imaging of single molecules in a solution" (emphasis added)). While Williams detects single label molecules in solution, it does not teach or suggest how a single molecule, bound to a solid support can be detected.

There is no motivation to combine Ross and Williams because the two methods of label detection are incompatible. As discussed above, Ross is directed to sequencing multiple identical templates which necessitated the detection of multiple labels simultaneously. In contrast, Williams is directed to sequencing individual DNA molecules and requires the detection of a single label in solution. Ross' multiple label detection method does not have the sensitivity required to detect the single label produced by Williams' sequencing procedure. Furthermore, Williams' method of detecting single molecules in solution would be incompatible with the multiple labels released at each sequencing cycle of Ross' method. Therefore, one of skill in the art would not combine the sequencing method of Ross and Williams because of these incompatibilities.

2. A combination of Ross and Williams would not lead to Applicants' Claimed Invention.

Ross and Williams, individually or in combination, do not disclose all the recitations of Applicants' claimed invention. Applicants' invention, as recited in amended claim 1, is directed to a method of sequencing where the sequence is determined by detecting a single labeling group which is attached to a solid support. This recitation is not disclosed or suggested by Ross, Williams or a combination of Ross and Williams.

As discussed above, Ross is directed to a method of sequencing involving the use of multiple identical templates DNA and the detection of multiple labels incorporated to primers attached to these template DNAs (Ross page 7 lines 1-14). The detection of a single label which is attached to a solid support, as recited in amended claim 1 of the instant application, is neither taught nor suggested by Ross. The addition of Williams does not cure this defect.

As discussed above, Williams characterized its sequencing technique as a method that "requires the imaging of single molecules in a solution" (Williams column 12, line 11-12, emphasis added). In Williams' method, nucleotides containing a quencher and a label, attached to the gamma phosphate, are used to elongate a nucleic acid template. The quencher inhibits fluorescence from the label because of its proximity to the label. Therefore, when the nucleotide is not incorporated, the quencher is proximal to the label and no fluoresces is observed. As the nucleotides are incorporated, the gamma phosphate is released from the nucleotide and released

from the growing DNA chain and detected (Williams, column 4 lines 4-22). Most importantly, unlike Applicant's claimed invention, Williams' gamma phosphate linked label is fluorescent only if it is released from the solid support because the quencher is attached to the solid support. Williams' labels, because of their proximity to the quencher, would not be detectable if they are attached to the solid support. Therefore, like Ross, Williams provides no suggestion or description of how a single label molecule attached to a solid support can be detected. Furthermore, the combination of Ross and Williams provide no support for the detection of a single molecule of label attached to a solid support - as required by Applicants' claimed invention.

For the reasons stated above, Williams, Ross, or a combination of Williams and Ross does not teach or suggest the detection of a single molecule of label attached to a solid support - which is a recitation of pending claim 1. Thus, the rejection of claims 1-13 in view of Ross and Williams is overcome and the withdrawal of the rejection is respectfully requested.

CONCLUSION

Favorable action on the merits is respectfully requested. If further discussion of this case is deemed helpful, the Examiner is encouraged to contact the undersigned at the telephone number provided below, and is assured of full cooperation in progressing the instant claims to allowance.

Applicants believe no further fee is due at this time; however, the Commissioner is authorized to charge any additional fees that may be due, or to credit any overpayment, to the undersigned's account, Deposit Account No. **50-0311**, Reference Number: **18921-001 NATL** (Customer Number: **35437**).

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